A1

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 9/90 — A1

(11) International Publication Number: **WO 96/40886**

(43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/09530

(22) International Filing Date: 7 June 1996 (07.06.96)

(30) Priority Data:

08/485,621	7 June 1995 (07.06.95)	US
60/000,399	21 June 1995 (21.06.95)	US

(60) Parent Applications or Grants
(63) Related by Continuation

US	08/485,621 (CIP)
Filed on	7 June 1995 (07.06.95)
US	60/000,399 (CIP)
Filed on	21 June 1995 (21.06.95)

(71) Applicant *(for all designated States except US)*: THOMAS JEFFERSON UNIVERSITY [US/US]; 11th and Walnut Streets, Philadelphia, PA 19107 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KMIEC, Eric, B. [US/US]; 1 Lapp Road, Malvern, PA 19355 (US). GERHOLD, David, L. [US/US]; 167 Meadowbrook Drive, Huntingdon Valley, PA 19006 (US). COLE STRAUSS, Allyson [US/US]; Apartment 1B, 515 South 9th Street, Philadelphia, PA 19147 (US).

(74) Agents: JOHNSON, Philip, S. et al.; Woodcock Washburn Kurtz Mackiewicz & Norris, 46th floor, One Liberty Place, Philadelphia, PA 19103 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ANTI-FUNGAL AGENTS AND METHODS OF IDENTIFYING AND USING THE SAME

(57) Abstract

Substantially pure *C. albicans* topoisomerase I protein is disclosed. Nucleic acid molecules that encode *C. albicans* topoisomerase I protein, recombinant expression vectors that comprise a nucleic acid sequence that encodes *C. albicans* topoisomerase I protein, and host cells that comprise recombinant expression vectors that comprise nucleic acid sequences that encode *C. albicans* topoisomerase I protein are disclosed. Fragments of nucleic acid molecules with sequences encoding *C. albicans* topoisomerase I protein and oligonucleotide molecules that comprise a nucleotide sequence complementary to fragment of a nucleotide sequence that encodes *C. albicans* topoisomerase I protein are disclosed. Antibodies which bind to an epitope on *C. albicans* topoisomerase I protein are disclosed. Methods of identifying inhibitors of *C. albicans* topoisomerase I protein are disclosed. Camptothecin analogs useful as inhibitors of *C. albicans* topoisomerase I protein are disclosed and methods of using camptothecin analogs as inhibitors of *C. albicans* topoisomerase I protein are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

ANTI-FUNGAL AGENTS AND METHODS OF IDENTIFYING AND USING THE SAME

FIELD OF THE INVENTION

The invention relates to the identification and cloning of the topoisomerase I gene (TOP1) from *Candida albicans* and the use of the gene in complementation assays to identify inhibitors of the *C. albicans* TOP1 while having no effect on the homologous human TOP1. The invention relates to compounds that selectively inhibit *C. albicans* TOP1 and the use of such compounds to kill fungi and in the treatment of individuals with fungal infections.

BACKGROUND OF THE INVENTION

Candida albicans is the most important fungal pathogen infecting humans. This fungal pathogen causes vaginal yeast infections, as well as oral infections and tissue invasion in immunocompromised patients. Oral infections are highly prevalent in AIDS patients and in cancer patients undergoing bone marrow replacement therapy. Only three types of anti-fungal drugs are currently approved for use in humans. Unfortunately, these anti-fungal drugs have serious side effects and have limited efficacy.

Yeast *Saccharomyces cerevisiae* strains that express DNA topoisomerase I and are permeable to the anti-tumor alkaloid camptothecin compounds are killed by the compound (Nitiss, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1988, *85*, 7501-7505). Yeast strains which are permeable to camptothecin but lack topoisomerase I can establish sensitivity to camptothecin by expression of human DNA topoisomerase I

(Bjornsti, *et al.*, *Cancer Res.*, 1989, *49*, 6318-6323). Thus, yeast cells lacking endogenous topoisomerase I are killed by camptothecin if they express human topoisomerase I. Camptothecin kills such yeast strains by stabilizing a covalent topoisomerase I-DNA conjugate which leaves a broken DNA strand. The broken single strand can be processed to a double-strand break during DNA replication. If this damage is not repaired by DNA recombination, it leads to cell death. Camptothecin, however, is not a candidate for human therapy for fungal-associated conditions due to its activity on human topoisomerase I.

There is a need for compounds which selectively inhibit *C. albicans* topoisomerase I activity but which do not inhibit human topoisomerase I activity. There is a need for kits and methods of identifying such compounds. There is a need for isolated *C. albicans* topoisomerase I protein, and for compositions and methods of producing and isolating *C. albicans* topoisomerase I protein. There is a need for methods of treating individuals that have fungal infections.

SUMMARY OF THE INVENTION

The present invention relates to substantially pure *C. albicans* topoisomerase I protein.

The present invention relates to substantially pure *C. albicans* topoisomerase I protein having the amino acid sequence of SEQ ID NO:2.

The present invention relates to nucleic acid molecules that encode *C. albicans* topoisomerase I protein.

The present invention relates to nucleic acid molecules encoding *C. albicans* topoisomerase I protein that consists of SEQ ID NO:1.

The present invention relates to recombinant expression vectors that comprise a nucleic acid sequence that encodes *C. albicans* topoisomerase I protein.

The present invention relates to host cells that comprise recombinant expression vectors that encode *C. albicans* topoisomerase I protein.

The present invention relates to fragments of nucleic acid molecules with sequences encoding *C. albicans* topoisomerase I protein that have at least 10 nucleotides.

The present invention relates to oligonucleotide molecules that comprise a nucleotide sequence complimentary to a nucleotide sequence of at least 10 nucleotides of SEQ ID NO:1.

The present invention relates to isolated antibodies which bind to an epitope on SEQ ID NO:2.

The present invention relates to host cells that have deficient or non-functional endogenous topoisomerase I proteins and comprise recombinant expression vectors that encode *C. albicans* topoisomerase I protein.

The present invention relates to methods of identifying inhibitors of *C. albicans* topoisomerase I protein. The methods comprise contacting a first host cell which is deficient in a functional topoisomerase gene except for a functional gene that encodes *C. albicans* topoisomerase I protein with a test compound, contacting a second host cell which is deficient in a functional topoisomerase gene except for a functional gene that encodes *non-C. albicans* topoisomerase I protein with a test compound, and identifying a test compound whose presence results in the death of the first host cell but not the second host cell.

The present invention relates to compounds that are specific inhibitors of *C. albicans* topoisomerase I protein which selective inhibit *C. albicans* topoisomerase I protein. The compounds of the invention inhibit *C. albicans* topoisomerase I protein much greater than they inhibit human or other non-*C. albicans* topoisomerase I protein such that the compounds of the invention are lethal to *C. albicans* through the inhibition of activity of *C. albicans* topoisomerase I protein but that do not kill non-*C. albicans* species which come into contact with the compound.

The present invention relates to compounds that are Camptothecin analogs which selectively inhibit *C. albicans* topoisomerase I. Camptothecin analogs of the invention

interact or otherwise interfere with the residues in the active site region of the *C. albicans* topoisomerase I, particularly the Methionine residue at Met736 which is present in *C. albicans* instead of the leucine located 2 residues amino-terminal to the active site tyrosine, Tyr738, found in human topoisomerase I.

The present invention relates to methods of treating individuals who have fungal infections comprising the step of administering to such individuals a therapeutically effective amount of a compound of the invention.

The present invention relates to methods of preventing fungal infections in individuals comprising the step of administering to such individuals a prophylactically effective amount of a compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides the cloned gene that encodes *C. albicans* topoisomerase I protein. The discovery of the *C. albicans* topoisomerase I gene and the protein that it encodes provides the means to design and discover specific inhibitors of *C. albicans* topoisomerase I protein.

As used herein the terms "specific inhibitor of *C. albicans* topoisomerase I protein" and "selective inhibitor of *C. albicans* topoisomerase I protein" are used interchangeably and are meant to refer to compounds that result in the death of *C. albicans* through the inhibition of activity of *C. albicans* topoisomerase I protein but that do not kill non-*C. albicans* species which come into contact with the compound. Compounds that selectively inhibit *C. albicans* topoisomerase I activity are those which inhibit *C. albicans* topoisomerase I activity but not the activity of non-*C. albicans* topoisomerase I proteins.

According to one aspect of the present invention, the gene that encodes *C. albicans* topoisomerase I protein may be used to produce recombinant microorganisms that are useful to screen compounds for specific inhibitors. A host

organism deficient in endogenous topoisomerase I protein may be "complemented" with *C. albicans* topoisomerase I, i.e. furnished with a functional copy of the *C. albicans* topoisomerase I gene or cDNA. Expression of the nucleotide sequence that encodes *C. albicans* topoisomerase I protein results in production of functional protein which functions in place of the missing or non-functional endogenous topoisomerase I. Comparative studies can be performed to evaluate the effect test compounds have on the hosts that are complemented with *C. albicans* topoisomerase I compared to the effect the same test compounds have on the hosts with functional endogenous topoisomerase I or hosts that are complemented with non-*C. albicans* topoisomerase I. In some preferred embodiments, inhibitors are identified using complementation assays in which a first host cell that expresses *C. albicans* topoisomerase I protein to survive is contacted with a test compound and a second host cell which expresses a non-*C. albicans* topoisomerase I protein to survive is contacted with the same test compound. If the first host cell dies in the presence of the test compound but the second host cell lives in the presence of the same test compound, the compound is indicated to be an inhibitor of *C. albicans* topoisomerase I protein.

Complemented host cells are deficient for functional endogenous topoisomerase I and rely on the activity of "foreign" topoisomerase I for survival. Host cells that are deficient for functional endogenous topoisomerase I and which can be complemented by "foreign" topoisomerase I for survival include yeasts, *Saccharomyces* species, *Schizosaccharomyces* species, *Escherichia coli*, and *Salmonella typhimurium*. In some preferred embodiments, complemented host cells are yeasts. In some preferred embodiments, complemented host cells are yeast strain L1242, which is described in Thrash, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1985, *82*, 4374-4378, which is disclosed in its entirety herein by reference, or other *top1*⁻ yeast strains.

Expression of human *TOP1* in yeast is described in

Bjornsti, *et al.*, *Cancer Res.*, 1989, *49*, 6318-6323, which is disclosed in its entirety herein by reference. Bjornsti, *et al.*, describe the complementation of conditional lethal human topoisomerase I mutant. In a similar manner, yeasts and the like can be transformed with nucleic acid molecules encoding *C. albicans* topoisomerase I protein that consists of SEQ ID NO:2. Accordingly, complementation can be performed with yeasts transformed with nucleic acid molecules encoding *C. albicans* topoisomerase I protein that consists of SEQ ID NO:2.

The methods of the invention are useful to identify selective inhibitors of *C. albicans* topoisomerase I protein. Inhibitors are useful as anti-fungal agents, specifically anti-*C. albicans* agents. Kits are provided for screening compounds for identifying selective inhibitors of *C. albicans* topoisomerase I protein.

The nucleotide sequence that encodes *C. albicans* topoisomerase I protein and that is disclosed herein as SEQ ID NO:1 allows for the production of complemented host cells which survive due to the presence of functional *C. albicans* topoisomerase I protein. In preparing gene constructs for complementation of deficient hosts, SEQ ID NO:1 is introduced into a host and expressed. SEQ ID NO:1 may be inserted into an expression vector in which the coding sequence is operably linked to regulatory elements required for gene expression in the host. In some preferred embodiments the expression vector is pBM272, which allows regulated expression from the GAL1 promoter of *Saccharomyces cerevisiae*. The wild-type *C. albicans* TOP1 coding sequence can be inserted into the BamHI and HindIII sites of pBM272. As controls, deficient host cells may be complemented with human topoisomerase I or another topoisomerase I.

The nucleotide sequence that encodes *C. albicans* topoisomerase I protein and that is disclosed herein as SEQ ID NO:1 allows for the production of pure *C. albicans* topoisomerase I protein and the design of probes which specifically hybridize to nucleic acid molecules that encode

C. albicans topoisomerase I protein and antisense compounds to inhibit transcription of the gene that encodes *C. albicans* topoisomerase I protein.

The present invention provides substantially purified *C. albicans* topoisomerase I protein. The present invention provides substantially purified *C. albicans* topoisomerase I protein which has the amino acid sequence consisting of SEQ ID NO:2. *C. albicans* topoisomerase I protein can be isolated from natural sources or produced by recombinant DNA methods.

The *C. albicans* topoisomerase I protein sequence differs substantially from the human topoisomerase I sequence. Such differences may be used to predict which compounds might show specific binding or inhibition of the *C. albicans* topoisomerase I. In particular, the active site region of the *C. albicans* topoisomerase I has a methionine residue, Met736, instead of the leucine/isoleucine located 2 residues amino-terminal to the active site tyrosine, Tyr738, found in human and other eukaryotic topoisomerase I proteins. Antibodies may be generated and selected which specifically bind to *C. albicans* topoisomerase I at an epitope which includes the methionine within the active site.

Antibodies that specifically bind to *C. albicans* topoisomerase I protein are provided. Such antibodies are specific inhibitors of *C. albicans* topoisomerase I protein and may be used in methods of isolating pure *C. albicans* topoisomerase I protein and methods of inhibiting *C. albicans* topoisomerase I protein activity.

The antibodies may be used to purify the protein from natural sources using well known techniques and readily available starting materials. Such antibodies may also be used to purify *C. albicans* topoisomerase I protein from material present when producing the protein by recombinant DNA methodology. The present invention relates to antibodies that bind to an epitope which is specific for *C. albicans* topoisomerase I protein as compared to human

topoisomerase I protein. This epitope appears at amino acids 730 to 740 of SEQ ID NO:2.

As used herein, the term "antibody" is meant to refer to complete, intact antibodies, and Fab fragments and $F(ab)_2$ fragments thereof. Complete, intact antibodies include monoclonal antibodies such as murine monoclonal antibodies, chimeric antibodies and humanized antibodies. The antibodies specifically bind to an epitope on SEQ ID NO:2. In some preferred embodiments, that epitope appears at amino acids 730 to 740 of SEQ ID NO:2. Antibodies that bind to an epitope on SEQ ID NO:2, particularly at amino acids 730 to 740 of SEQ ID NO:2 are useful to isolate and purify *C. albicans* topoisomerase I protein from both natural sources or recombinant expression systems using well known techniques such as affinity chromatography. Such antibodies are useful to detect the presence of such protein in a sample and to determine if cells are expressing the protein.

The production of antibodies and the protein structures of complete, intact antibodies, Fab fragments and $F(ab)_2$ fragments and the organization of the genetic sequences that encode such molecules are well known and are described, for example, in Harlow, E. and D. Lane (1988) *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, which is incorporated herein by reference. Briefly, for example, the *C. albicans* topoisomerase I protein, or an immunogenic fragment thereof is injected into mice. The spleen of the mouse is removed, the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, or hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to *C. albicans* topoisomerase I protein, the hybridoma which produces them is cultured to produce a continuous supply of antibodies.

Using standard techniques and readily available starting materials, a nucleic acid molecule that encodes *C. albicans* topoisomerase I protein may be isolated from a cDNA

library, using probes which are designed using the nucleotide sequence information disclosed in SEQ ID NO:1. The present invention relates to an isolated nucleic acid molecule that comprises a nucleotide sequence that encodes *C. albicans* topoisomerase I protein and that comprises the amino acid sequence of SEQ ID NO:2. In some embodiments, the nucleic acid molecules consist of a nucleotide sequence that encodes *C. albicans* topoisomerase I protein. In some embodiments, the nucleic acid molecules comprise the nucleotide sequence that consists of the coding sequence in SEQ ID NO:1. In some embodiments, the nucleic acid molecules consist of the nucleotide sequence set forth in SEQ ID NO:1. The isolated nucleic acid molecules of the invention are useful to prepare constructs and recombinant expression systems for preparing isolated *C. albicans* topoisomerase I protein.

A genomic or cDNA library may be generated by well known techniques. Clones are identified using probes that comprise at least a portion of the nucleotide sequence disclosed in SEQ ID NO:1. The probes have at least 16 nucleotides, preferably 24 nucleotides. The probes are used to screen the genomic or cDNA libraries using standard hybridization techniques. In addition, the probes of the invention may be used to identify topoisomerase I genes from related organisms such as *Aspergillus fumigatus* and *Cryptosporidium* species.

The present invention relates to isolated nucleic acid molecules that comprise a nucleotide sequence identical or complementary to a fragment of SEQ ID NO:1 which is at least 10 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of a nucleotide sequence identical or complementary to a fragment of SEQ ID NO:1 which is at least 10 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consist of a nucleotide sequence identical or complementary to a fragment of SEQ ID NO:1 which is 15-150 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or

consist of a nucleotide sequence identical or complementary to a fragment of SEQ ID NO:1 which is 15-30 nucleotides.

Isolated nucleic acid molecules that comprise or consist of a nucleotide sequence identical or complementary to a fragment of SEQ ID NO:1 which is at least 10 nucleotides are useful as probes for identifying genes and cDNA sequences that encodes *C. albicans* topoisomerase I protein, PCR primers for amplifying genes and cDNA that encodes *C. albicans* topoisomerase I protein, and antisense molecules for inhibiting transcription and translation of genes and cDNA, respectively, which encode *C. albicans* topoisomerase I protein.

The nucleotide sequence in SEQ ID NO:1 may be used to design probes, primers and complimentary molecules which specifically hybridize to the unique nucleotide sequences of *C. albicans* topoisomerase I protein. Probes, primers and complimentary molecules which specifically hybridize to nucleotide sequence that encodes *C. albicans* topoisomerase I protein may be designed routinely by those having ordinary skill in the art.

The present invention also includes labeled oligonucleotides which are useful as probes for performing oligonucleotide hybridization methods to identify clones that encode *C. albicans* topoisomerase I protein. Accordingly, the present invention includes probes that can be labelled and hybridized to unique nucleotide sequences of nucleic acid molecules that encode *C. albicans* topoisomerase I protein. The labelled probes of the present invention are labelled with radiolabeled nucleotides or are otherwise detectable by readily available nonradioactive detection systems. In some preferred embodiments, probes comprise oligonucleotides consisting of between 10 and 100 nucleotides. In some preferred, probes comprise oligonucleotides consisting of between 10 and 50 nucleotides. In some preferred, probes comprise oligonucleotides consisting of between 12 and 20 nucleotides. The probes preferably contain nucleotide

sequence completely identical or complementary to a fragment of a unique nucleotide sequences of nucleic acid molecules that encode *C. albicans* topoisomerase I protein.

PCR technology is practiced routinely by those having ordinary skill in the art and its uses in diagnostics are well known and accepted. Methods for practicing PCR technology are disclosed in "PCR Protocols: A Guide to Methods and Applications", Innis, M.A., *et al.* Eds. Academic Press, Inc. San Diego, CA (1990), which is incorporated herein by reference. Applications of PCR technology are disclosed in "Polymerase Chain Reaction" Erlich, H.A., *et al.*, Eds. Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), which is incorporated herein by reference. Some simple rules aid in the design of efficient primers. Typical primers are 18-28 nucleotides in length having 50% to 60% g+c composition. The entire primer is preferably complementary to the sequence it must hybridize to. Preferably, primers generate PCR products 100 base pairs to 2000 base pairs. However, it is possible to generate products of 50 base pairs to up to 10 kb and more.

PCR technology allows for the rapid generation of multiple copies of nucleotide sequences by providing 5' and 3' primers that hybridize to sequences present in a nucleic acid molecule, and further providing free nucleotides and an enzyme which fills in the complementary bases to the nucleotide sequence between the primers with the free nucleotides to produce a complementary strand of DNA. The enzyme will fill in the complementary sequences adjacent to the primers. If both the 5' primer and 3' primer hybridize to nucleotide sequences on the complementary strands of the same fragment of nucleic acid, exponential amplification of a specific double-stranded product results. If only a single primer hybridizes to the nucleic acid molecule, linear amplification produces single-stranded products of variable length.

One having ordinary skill in the art can isolate the nucleic acid molecule that encodes *C. albicans*

topoisomerase I protein and insert it into an expression vector using standard techniques and readily available starting materials. The present invention relates to a recombinant expression vector that comprises a nucleotide sequence that encodes *C. albicans* topoisomerase I protein that comprises the amino acid sequence of SEQ ID NO:2. As used herein, the term "recombinant expression vector" is meant to refer to a plasmid, phage, viral particle or other vector which, when introduced into an appropriate host, contains the necessary genetic elements to direct expression of the coding sequence that encodes the *C. albicans* topoisomerase I protein. The coding sequence is operably linked to the necessary regulatory sequences. Expression vectors are well known and readily available. Examples of expression vectors include plasmids, phages, viral vectors and other nucleic acid molecules or nucleic acid molecule containing vehicles useful to transform host cells and facilitate expression of coding sequences. In some embodiments, the recombinant expression vector comprises the nucleotide sequence set forth in SEQ ID NO:1. The recombinant expression vectors of the invention are useful for transforming hosts to prepare recombinant expression systems for preparing the *C. albicans* topoisomerase I protein.

The present invention relates to a host cell that comprises the recombinant expression vector that includes a nucleotide sequence that encodes *C. albicans* topoisomerase I protein that comprises SEQ ID NO:2. In some embodiments, the host cell comprises a recombinant expression vector that comprises SEQ ID NO:1. Host cells for use in well known recombinant expression systems for production of proteins are well known and readily available. Examples of host cells include bacteria cells such as *E. coli*, yeast cells such as *S. cerevisiae*, insect cells such as *S. frugiperda*, non-human mammalian tissue culture cells chinese hamster ovary (CHO) cells and human tissue culture cells such as HeLa cells.

The present invention relates to a transgenic, non-human mammal that comprises the recombinant expression vector that comprises a nucleic acid sequence that encodes the *C. albicans* topoisomerase I protein that comprises the amino acid sequence of SEQ ID NO:2. Transgenic, non-human mammals useful to produce recombinant proteins are well known as are the expression vectors necessary and the techniques for generating transgenic animals. Generally, the transgenic animal comprises a recombinant expression vector in which the nucleotide sequence that encodes *C. albicans* topoisomerase I protein operably linked to a mammary cell specific promoter whereby the coding sequence is only expressed in mammary cells and the recombinant protein so expressed is recovered from the animal's milk. In some embodiments, the coding sequence that encodes *C. albicans* topoisomerase 1 protein is SEQ ID NO:1.

In some embodiments, for example, one having ordinary skill in the art can, using well known techniques, insert such DNA molecules into a commercially available expression vector for use in well known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, CA) may be used for production of *C. albicans* topoisomerase I in *E. coli*. The commercially available plasmid pYES2 (Invitrogen, San Diego, CA) may, for example, be used for production in *S. cerevisiae* strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, CA) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA I (Invitrogen, San Diego, CA) may, for example, be used for production in mammalian cells such as CHO cells. One having ordinary skill in the art can use these commercial expression vectors and systems or others to produce *C. albicans* topoisomerase I protein using routine techniques and readily available starting materials. (*See e.g.*, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989), which is incorporated herein by reference.)

Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

One having ordinary skill in the art may use other commercially available expression vectors and systems or produce vectors using well known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers, are readily available and known in the art for a variety of hosts. *See e.g.*, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989).

A wide variety of eukaryotic hosts are also now available for production of recombinant foreign proteins. As in bacteria, eukaryotic hosts may be transformed with expression systems which produce the desired protein directly, but more commonly signal sequences are provided to effect the secretion of the protein. Eukaryotic systems have the additional advantage that they are able to process introns which may occur in the genomic sequences encoding proteins of higher organisms. Eukaryotic systems also provide a variety of processing mechanisms which result in, for example, glycosylation, carboxy-terminal amidation, oxidation or derivatization of certain amino acid residues, conformational control, and so forth.

Commonly used eukaryotic systems include, but is not limited to, yeast, fungal cells, insect cells, mammalian cells, avian cells, and cells of higher plants. Suitable promoters are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, e.g. the baculovirus polyhedron promoter. As above, promoters can be either constitutive or inducible. For example, in mammalian systems, the mouse metallothionein promoter can be induced by the addition of heavy metal ions.

The particulars for the construction of expression systems suitable for desired hosts are known to those in the art. Briefly, for recombinant production of the protein, the DNA encoding the polypeptide is suitably ligated into the expression vector of choice. The DNA is operably linked to all regulatory elements which are necessary for expression of the DNA in the selected host. One having ordinary skill in the art can, using well known techniques, prepare expression vectors for recombinant production of the polypeptide.

The expression vector including the DNA that encodes *C. albicans* topoisomerase I protein is used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign DNA takes place. The protein of the present invention thus produced is recovered from the culture, either by lysing the cells or from the culture medium as appropriate and known to those in the art. One having ordinary skill in the art can, using well known techniques, isolate *C. albicans* topoisomerase I protein that is produced using such expression systems. The methods of purifying *C. albicans* topoisomerase I protein from natural sources using antibodies which specifically bind to *C. albicans* topoisomerase I protein as described above, may be equally applied to purifying *C. albicans* topoisomerase I protein produced by recombinant DNA methodology.

Examples of genetic constructs include the *C. albicans* topoisomerase I protein coding sequence operably linked to a promoter that is functional in the cell line into which the constructs are transfected. Examples of constitutive promoters include promoters from cytomegalovirus or SV40. Examples of inducible promoters include mouse mammary leukemia virus or metallothionein promoters. Those having ordinary skill in the art can readily produce genetic constructs useful for transfecting with cells with DNA that encodes *C. albicans* topoisomerase I protein from readily available starting materials. Such

gene constructs are useful for the production of *C. albicans* topoisomerase I protein.

In some embodiments of the invention, transgenic non-human animals are generated. The transgenic animals according to the invention contain SEQ ID NO:1 under the regulatory control of a mammary specific promoter. One having ordinary skill in the art using standard techniques, such as those taught in U.S. Patent No. 4,873,191 issued October 10, 1989 to Wagner and U.S. Patent No. 4,736,866 issued April 12, 1988 to Leder, both of which are incorporated herein by reference, can produce transgenic animals which produce the *C. albicans* topoisomerase I protein. Preferred animals are rodents, particularly goats, rats and mice.

In addition to producing these proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce *C. albicans* topoisomerase I protein. Such techniques are well known to those having ordinary skill in the art and are useful if derivatives which have substitutions not provided for in DNA-encoded protein production.

To screen compounds according to the methods of the present invention, at least two groups of host cells are tested. One host cell is complemented with functional *C. albicans* topoisomerase I. The other host cell either contains a functional endogenous topoisomerase I or is complemented with a non-*C. albicans* topoisomerase, preferably human topoisomerase. The groups are contacted with test compounds and the survivability of each of the two groups is observed. If a test compound leads to the death of the host cells complemented with *C. albicans* topoisomerase I but not those with non-*C. albicans* topoisomerase I, the compound is a selective inhibitor of *C. albicans* topoisomerase I.

In some embodiments of the invention, the preferred concentration of test compound is between 1μM and 500μM. A preferred concentration is 10μM to 100μM. In some preferred

embodiments, it is desirable to use a series of dilutions of test compounds.

Kits are included which comprise containers with host cells or reagents necessary to produce host cells and/or screen test compounds. In additions, kits comprise instructions for performing such methods.

Another aspect of the present invention relates to camptothecin analogs that selectively inhibit *C. albicans* topoisomerase I protein, but not human topoisomerase I. According to the present invention, such compounds may be administered to individuals identified as suffering from fungal infections to kill the infecting organism.

Camptothecin analogs which selectively inhibit *C. albicans* topoisomerase I protein may be identified using the assay of the present invention. The host organism deficient in endogenous topoisomerase I protein is complemented with *C. albicans* topoisomerase I and comparative studies are performed to evaluate the effect that camptothecin analogs have on the hosts that are complemented with *C. albicans* topoisomerase I compared to the effect the same camptothecin analog has on the hosts with functional endogenous topoisomerase I or hosts that are complemented with non-*C. albicans* topoisomerase I. In some preferred, camptothecin analogs that selectively inhibit *C. albicans* topoisomerase I are identified using complementation assays in which a first host cell that expresses *C. albicans* topoisomerase I protein to survive is contacted with a camptothecin analog and a second host cell which expresses a non-*C. albicans* topoisomerase I protein to survive is contacted with the same camptothecin analog. If the first host cell dies in the presence of the camptothecin analog but the second host cell lives in the presence of the same camptothecin analog, the camptothecin analog is indicated to be a selective inhibitor of *C. albicans* topoisomerase I protein.

The assay of the invention is useful to identify camptothecin analogs that are selective inhibitors of *C. albicans* topoisomerase I protein. The camptothecin analogs

that are selective inhibitors are useful as anti-fungal agents, specifically anti-*C. albicans* agents.

In some embodiments, compounds of the invention interact with Met736.

The invention relates to camptothecin analogs that are selective inhibitors of *C. albicans* topoisomerase I. Camptothecin analogs that are selective inhibitors of *C. albicans* topoisomerase I may be identified by screening camptothecin analogs disclosed in U.S. Patent Number 5,405,963; U.S. Patent Number 5,401,747; U.S. Patent Number 5,395,939; U.S. Patent Number 5,391,745; U.S. Patent Number 5,364,858; U.S. Patent Number 5,342,947; U.S. Patent Number 5,244,903; U.S. Patent Number 5,227,380; U.S. Patent Number 5,223,506; U.S. Patent Number 5,212,317; U.S. Patent Number 5,200,524; U.S. Patent Number 5,191,082; U.S. Patent Number 5,180,722; U.S. Patent Number 5,162,532; U.S. Patent Number 5,155,225; U.S. Patent Number 5,122,606; U.S. Patent Number 5,122,526; U.S. Patent Number 5,106,742; U.S. Patent Number 5,061,800; U.S. Patent Number 5,061,795; U.S. Patent Number 5,053,512; U.S. Patent Number 5,041,424; U.S. Patent Number 5,004,758; U.S. Patent Number 4,981,968; U.S. Patent Number 4,939,255; U.S. Patent Number 4,914,205; U.S. Patent Number 4,604,463; U.S. Patent Number 4,545,880; U.S. Patent Number 4,513,138; U.S. Patent Number 4,473,692; U.S. Patent Number 4,399,282; U.S. Patent Number 4,399,276; U.S. Patent Number 4,031,098; and U.S. Patent Number 3,894,029; which are each incorporated herein by reference. The present invention relates to camptothecin analogs disclosed in the patents which are inhibitors of *C. albicans*.

In some embodiments of the invention, the preferred concentration of camptothecin analogs is between 1μM and 500μM. A preferred concentration is 10μM to 100μM. In some preferred embodiments, it is desirable to use a series of dilutions of test compounds.

The present invention relates to methods of inhibiting *C. albicans* topoisomerase I activity which comprises contacting *C. albicans* topoisomerase I with an

effective amount of a camptothecin analog with selective inhibitory activity, or its pharmaceutically acceptable salt. Camptothecin analogs that are *C. albicans* topoisomerase I inhibitors are useful as antifungal compounds. The present invention relates to methods of treating an animal suffering from a fungal infection by administering an amount of a *C. albicans* topoisomerase I with an effective amount of a camptothecin analog or analogs with selective inhibitory activity, or its pharmaceutically acceptable salt, effective to inhibit *C. albicans* topoisomerase I activity.

The method that is the present invention is useful in the treatment of diseases which involve fungal infections such as opportunistic infections in immunocompromised patients such as those suffering from HIV infection including those having AIDS. In addition, the methods are useful for treating vaginal yeast infections. Accordingly, the present invention relates to a method of treating a mammal suffering from a fungal infection that comprises administering to the mammal, a therapeutically effective amount of a camptothecin analog with selective inhibitory activity, or its pharmaceutically acceptable salt which inhibits *C. albicans* topoisomerase I. Therapeutically effective amounts of compounds used in the method that is the present invention can be formulated as pharmaceutical preparations and administered to mammals who are suffering from fungal infections in order to counter the infection.

The method that is the present invention is useful in the prevention of fungal infections such as opportunistic infections in immunocompromised patients such as those suffering from HIV infection including those having AIDS. In addition, the methods are useful for treating vaginal yeast infections. Accordingly, the present invention relates to a method of administering a prophylactically effective amount of a camptothecin analog with selective inhibitory activity, or its pharmaceutically acceptable salt which inhibits *C. albicans* topoisomerase I to a mammal

susceptible to fungal infection. Individuals susceptible to fungal infections include immunocompromised individuals prone to opportunistic infections such as individuals suffering from HIV infection including those with AIDS or patients undergoing intensive radiation and/or chemotherapies that result in an reduction in the resistance to infection, or women undergoing therapy that includes antibiotics. A prophylactically effective dose is one in which the incidence of fungal infection is decreased upon administration of such a dose compared to the incidence of fungal infection which would occur in the absence of such a dose.

Pharmaceutically acceptable salts of these compounds may be used in practicing the methods that are the present invention. Pharmaceutical compositions containing the compounds or salts may also be used in practicing the methods that are the present invention. Pharmaceutically acceptable salts useful in the methods of that are the invention include sodium, potassium, calcium, zinc, lithium, magnesium, aluminum, diethanolamine, tromethamine, ethylenediamine, meglumine, hydrochloric, hydrobromic or acetic acid.

The present invention relates to a method of using a camptothecin analog with selective inhibitory activity, or its pharmaceutically acceptable salt which inhibits *C. albicans* topoisomerase I to inhibit the activity of *C. albicans* topoisomerase I in cells. The range of amounts of camptothecin analog with selective inhibitory activity, or its pharmaceutically acceptable salt that a cell can be exposed to and be effective for inhibiting *C. albicans* topoisomerase I can be determined by one having ordinary skill in the art.

By inhibiting *C. albicans* topoisomerase I activity, the method that is the present invention is useful in the treatment and/or prevention of fungal infections.

The mode of administration of compounds and pharmaceutical compositions according to the methods that

are the invention includes any means that produces contact of the active ingredient with the infectious organism in the body of a mammal or in a body fluid or tissue. These modes of administration include but not limited to oral, topical, hypodermal, intravenous, intramuscular and intraparenteral methods of administration. In practicing the methods that are the invention, the compounds may be administered singly or in combination with other compounds used in the methods of the invention, other pharmaceutical compounds, or in conjunction with therapies. In the methods of the invention, the compounds are preferably administered with a pharmaceutically acceptable carrier selected on the basis of the selected route of administration and standard pharmaceutical practice. One camptothecin analog or a plurality of camptothecin analogs in combination may be administered.

The methods may include administration of compounds to mammals, preferably humans, in therapeutically effective amounts which are effective to inhibit *C. albicans* topoisomerase I and kill *C. albicans*. The dosage administered in any particular instance will depend upon factors such as the pharmacodynamic characteristics of the compound of the invention, its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment, and the effect desired.

It is contemplated that the daily dosage of a compound used in the methods of the invention will be in the range of from about 1 μg to about 100 mg per kg of body weight, preferably from about 1 μg to about 40 mg per kg body weight, more preferably from about 10 μg to about 20 mg per kg per day, and most preferably 10 μg to about 1 mg per kg per day. Pharmaceutical compositions may be administered in a single dosage, divided dosages or in sustained release. Persons of ordinary skill will be able to determine dosage forms and amounts with only routine experimentation based upon the considerations of this invention. Isomers of the

compounds and pharmaceutical compositions, particularly optically active stereoisomers, are also within the scope of the present invention.

Compounds may be administered as pharmaceutical compositions orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The compounds may also be administered parenterally in sterile liquid dosage forms or topically in a carrier. The compounds may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See *Remington's Pharmaceutical Sciences*, A. Osol, Mack Publishing Company, Easton, Pennsylvania.

Compounds may be mixed with powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, and stearic acid for insertion into gelatin capsules, or for forming into tablets. Both tablets and capsules may be manufactured as sustained release products for continuous release of medication over a period of hours. Compressed tablets can be sugar or film coated to mask any unpleasant taste and protect the tablet from the atmosphere or enteric coated for selective disintegration in the gastrointestinal tract.

Liquid dosage forms for oral administration may contain coloring and flavoring to increase patient acceptance, in addition to a pharmaceutically acceptable diluent such as water, buffer or saline solution.

For parenteral administration, a compound may be mixed with a suitable carrier or diluent such as water, a oil, saline solution, aqueous dextrose (glucose), and related sugar solutions, and glycols such as propylene glycol or polyethylene glycols. Solutions for parenteral administration contain preferably a water soluble salt of the compound. Stabilizing agents, antioxidizing agents and preservatives may also be added. Suitable antioxidizing agents include sodium bisulfite, sodium sulfite, and ascorbic acid, citric acid and its salts, and sodium EDTA.

Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol.

Examples

Example 1

The topoisomerase I gene (*TOP1*) from *Candida albicans* is highly expressed in a yeast (*Saccharomyces cerevisiae*) strain lacking its native yeast *TOP1* gene. The human *TOP1* gene is highly expressed in a second *top1*-yeast strain. These two yeast strains are used to screen chemical compounds to find compounds which kill or inhibit the yeast expressing *C. albicans TOP1* but not the yeast expressing the human *TOP1*.

C. albicans TOP1 was cloned using PCR. The PCR fragment was used as a probe to select a full-length *TOP1* clone. The DNA sequence of the *C. albicans TOP1* gene was determined and used to predict the topoisomerase I peptide sequence. The gene was excised from the DNA library vector using restriction enzymes, modified at the start of the protein-coding sequence, and ligated into a yeast expression plasmid. This plasmid is transformed into a *top1*⁻ yeast strain.

The *C. albicans* topoisomerase I protein sequence differs substantially from the human topoisomerase I sequence. Such differences may be used to predict which compounds might show specific binding or inhibition of the *C. albicans* topoisomerase I. In particular, the active site region has a methionine residue in place of the usual leucine/isoleucine located 2 residues amino-terminal to the active site tyrosine. Drugs may be selected based on ability to interact with this methionine residue.

It has been shown previously that overexpression of a *TOP1* gene from another organism sensitizes a host yeast strain to camptothecin. Camptothecin kills such yeast strains by stabilizing a covalent topoisomerase I-DNA conjugate which leaves a broken DNA strand. The broken single strand can be processed to a double-strand break

during DNA replication. If this damage is not repaired by DNA recombination, it leads to cell death.

The fastest ways to screen chemical or natural compounds or extracts, such as camptothecin analogs, for activity against *C. albicans* topoisomerase I is an adaption of the "zone of inhibition" assay for antibiotics. Two yeast strains, one expressing *C. albicans* topoisomerase I and the other strain expressing human topoisomerase I, are spread into a lawn of cells on minimal medium in 2 petri-dishes. Duplicate small paper discs are soaked in solutions of chemicals or natural products, and transferred to the surfaces of each of the 2 petri dishes. After 2-4 days at 30°, a thick lawn of yeast cells will grow on the petri-dishes. A compound, such as an active camptothecin analog, which produces a clear "zone of inhibition" of growth on the *C. albicans TOP1* dish, but not the human *TOP1* dish, is a specific inhibitor of *C. albicans* topoisomerase I.

In another embodiment of the assay, the two yeast strains, one expressing *C. albicans* topoisomerase I and the other expressing human topoisomerase I, are grown in liquid medium containing a possible inhibitory agent. A compound that inhibits the growth of *C. albicans TOP1* yeast strain, but not the human *TOP1* yeast strain, is a specific inhibitor of the *C. albicans* topoisomerase I.

Example 2

Yeast Transformation

The plasmid pBM-CaTOP1, and a similar plasmid expressing the human TOP1 gene, can be transformed into a *top1⁻ S. cerevisiae* strain by standard techniques, such as those described in Elble, R., *Biotechniques*, 1992, *13(1)*, 78-80, which is disclosed in its entirety herein by reference. The plasmid can be selected by growing the yeast strain in minimal medium lacking uracil. The *URA3* gene within pBM-CaTOP1 will enable the yeast strain to grow on medium lacking uracil. The expression of *C. albicans* topoisomerase I in *S. cerevisiae* can be verified by assaying the ability of a crude extract of this yeast strain to

remove plasmid DNA supercoils as detailed in Thrash, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1985, *82*, 4374-4378, which is disclosed in its entirety herein by reference.

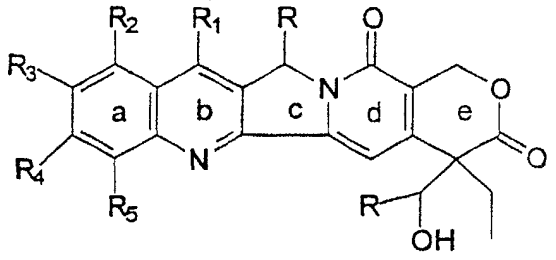
Cloning C. albicans TOP1 Gene Into Expression Vector pBM272

The native genomic *C. albicans TOP1* clone pCaT1-R12 constitutes a 3.4 kb EcoRI-EcoRI fragment containing the entire *TOP1* gene, ligated into the pBC SK(-) plasmid (Stratagene, La Jolla, CA). This gene was modified by introducing a BamHI restriction site immediately 5' upstream of the coding sequence using standard techniques (Sambrook, *et al.*, *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989)). The entire gene coding sequence was excised as a 2.4 kb BamHI-HindIII DNA fragment, and ligated into the BamHI-HindIII sites of yeast expression vector pBM272 using standard techniques. This plasmid, pBM-*CaTOP1*, can be introduced into *top1*⁻ yeast strain L1242 (*S. cerevisiae*; Thrash, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1985, *82*, 4374-4378) or a derivative strain, K2979, provided by Dr. Ralph Keil, Hershey Medical Center, Hershey, PA). The K2979 genotype is: MATa HindIII(*top1*::LEU2) *his4-260 ade2-1 ura3-52 leu2-3,112 trp1-HIII can1*^{R} *lys2*ΔBX::CAN1::LYS2 rDNA::URA3 rDNA::ADE2.

The cloned genomic *C. albicans TOP1* gene can also be used to generate a *top1*⁻/*top1*⁻ *C. albicans* strain using the gene for gene disruption using standard techniques known to fungal geneticists.

Example 3

Camptothecin analogs may have the formula:



wherein:

R is a lower alkyl;

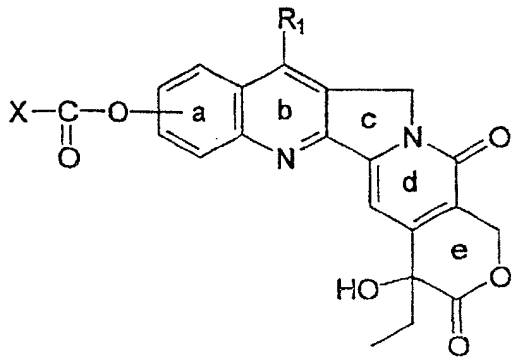
R_1 is H, lower alkyl, lower alkoxy, or halo; and

R_2, R_3, R_4 and R_5 may each independently be H, amino hydroxy, lower alkyl, lower alkoxy, lower alkylthio, di(lower alkyl)amino, cyano, methylenedioxy, Formyl, nitro, halo, trifluoromethyl, aminomethyl, azido, amido, hydrazino, or any of the standard twenty amino acids bonded to the A ring via the amino-nitrogen atom.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 5,212,317, U.S. Patent Number 5,191,082, U.S. Patent Number 5,395939, U.S. Patent Number 5,162,532, and U.S. Patent Number 5,200,524.

Example 4

Camptothecin analogs may have the formula:



wherein:

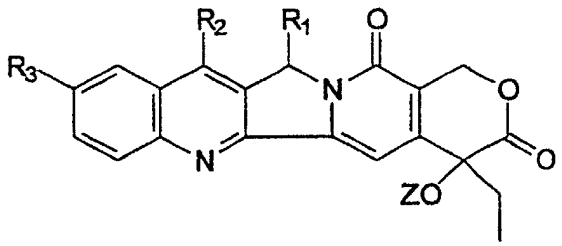
R_1 is H, halo, or an alkyl group with 1-4 carbons; and

X is a chlorine atom or $-NR_2R_3$ where R_2 and R_3 are the same or different and each represents a hydrogen, a substituted or unsubstituted alkyl group with 1-4 carbons, or a substituted or unsubstituted carbocyclic or heterocyclic group.

The synthesis of Camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 4,604,463.

Example 5

Camptothecin analogs may have the formula:



wherein:

R_1 is H, alkyl, hydroxyl, CH_2OH, COOH, aralkyl, alkoxy, acyloxy, CH_2OR_4, $COOR_5$ wherein R_4 is an alkyl or acyl group and R_5 is a lower alkyl or acyl group;

R_2 is H, alkyl, aralkyl, hydroxymethyl, carboxymethyl, acyloxymethyl, -CHO, $-CH_2OR'$, $-CH(OR')_2$ or -CH=N-X where R' is a lower alkyl group with 1-6 carbons or a phenylalkyl group with 1-3 carbon in the alkylene moiety thereof and X is hydroxyl or $-NR_6R_7$ where R_6 and R_7 are the same or different and each represents a hydrogen, or an alkyl group with 1-6 carbons, or when R_6 is hydrogen, R_7 may be an alkyl group with 1-6 carbons, a substituted or unsubstituted aryl group, a carbamoyl group, an acyl group, an aminoalkyl group or an amido group or when R_6 is a lower

alkyl group R_7 may be an aminoalkyl group or R_6 and R_7 may be combined together with a nitrogen to form a heterocycle or quaternary salt thereof;

R_3 is the grouping -XR' where R' is H, alkyl, or acyl and X is oxygen or sulphur, a nitro group, an amino group, an alkylamino group, an acylamino group, or a halogen; and

Z is hydrogen or an acyl group.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 4,473,692, U.S. Patent Number 4,545,880, 4,399,276, 4,399,282.

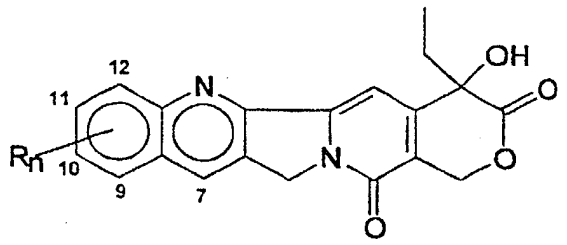
Example 6

Camptothecin analogs may be camptothecin oxide derivatives which have the formula as shown in Example 5 except that the Nitrogen on ring b is substituted with an oxygen.

The synthesis of camptothecin oxide derivatives with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 4,513,138.

Example 7

Camptothecin analogs may have the formula:



wherein ring a is substituted by R_n. Substituents include hydroxy, nitro, amino, chloro, bromo, iodo, fluoro, C_{1-8} alkyl, C_{1-8} alkoxy, trifluoromethyl, amino methyl, amido, hydrazino, azido, formyl,and cyano groups as well as groups

comprising amino acids bonded to the A ring via the amino-nitrogen atom. Preferred alkyl groups include methyl, ethyl, propyl, butyl, isopropyl, isbutyl and secbutyl groups. Preferred alkoxy groups include methoxy, ethoxy, propoxy, and isopropoxy groups. Preferred amino acids are the the standard twenty amino acids. Two substituents on the a ring may be joined together to form a bifunction substituent. In addition, ring a may be modified to contain a hetero atom. Ring a may be a five or six carbon ring with an oxygen, nitrogen or sulphur.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 5,106,742, U.S. Patent Number 5,122,526, U.S. Patent Number 4,981,968, U.S. Patent Number 5,180,722, U.S. Patent Number 5,401,747, U.S. Patent Number 5,227,380, U.S. Patent Number 5,364,858, U.S. Patent Number 5,244,903.

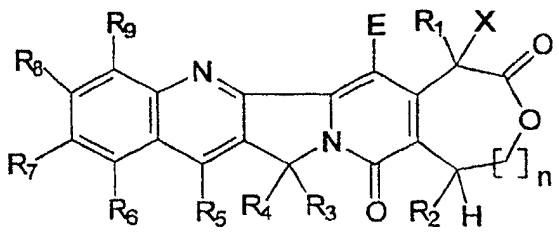
Example 8

Camptothecin analogs which have the formula as shown in Example 7 may be further substituted at the carbon on ring b *ortho* to the nitrogen on ring b. The position may substituted with a C_{1-8} alkyl.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 5,122,606 and U.S. Patent Number 5,053,512.

Example 9

Camptothecin analogs may have the formula:



wherein:

E is H, CO_2R, $CONH_2$, CONHR, $CONR_2$, acyl, or CN;

X is H, OH, or OR;

R_1, R_2, R_3, and R_4 are independently the same or different and are H, or a linear or branched chain alkyl, alkylaryl, hydroxyalkyl group, or an aryl group, and R_1 may be allyl, propargyl or benzyl;

R_5, R_6, R_7, R_8, and R_9, are independently the same or different and are H, or a linear or branched chain alkyl, alkylaryl, alkoxy, hydroxyalkyl group, or aminoalkoxy group, or an aryl or aryloxy group, or an amino, lower acylamino, di(lower alkyl)amino group, or a C-glycal or hydroxyl, CO_2R, nitro, cyano, Cl, F, Br, I, SR_{10}, $NR_{11}R_{12}$ or OR_{13}, or R_6 is CHO, CH_2R_{14} and R_7 is H, hydroxy, $-CH_2NH_2$ or formyl;

R is H, or a linear or branched alkyl, alkylaryl, or hydroxyalkyl group, or an aryl group,

R_{10}, R_{11}, and R_{12}, are independently the same or different and are H, or a linear or branched chain alkyl, alkylaryl, hydroxyalkyl, or acyl group, or an aryl group;

R_{13} is glycosyl;

R_{14} is OR_{15}, SR_{15}, CH_2NH_2, cyano, $NR_{15}R_{16}$, or $N^+[R_{15}R_{16}R_{17}]$;

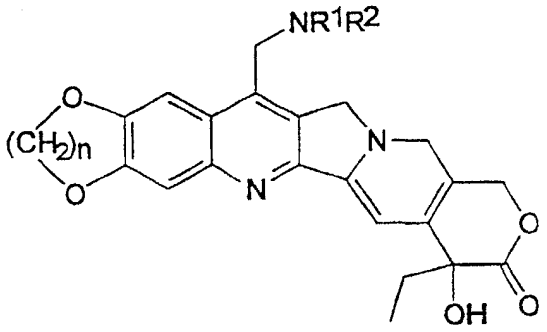
R_{15}, R_{16} and R_{17} are the same or different and are selected from H, C_{1-6} alkyl, C_{2-6} hydroxyalkyl, C_{1-6} dialkylamino-C_{2-6} alkyl, C_{1-6} alkylamino-C_{2-6} alkyl, C_{2-6} aminoalkyl or a 3-7 member unsubstituted carbocyclic ring; and

n is 0 or 1.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 5,391,745, U.S. Patent Number 5,061,800, U.S. Patent Number 5,004,758, U.S. Patent Number 4,031,098, and U.S. Patent Number 3,894,029.

Example 10

Camptothecin analogs may have the formula:



wherein:

n is 1 or 2;

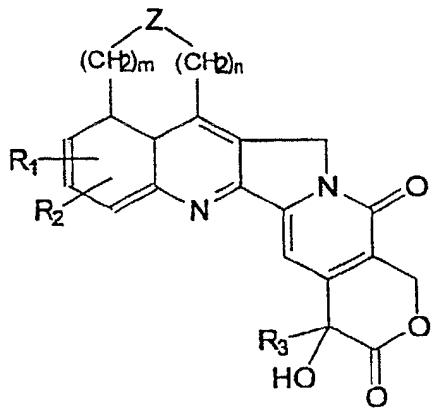
R_1 is independently, H, lower alkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, lower alkoxy lower alkyl; and

R_2 is H or a pharmaceutically acceptable salt.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 5,342,947.

Example 11

Camptothecin analogs may have the formula:



wherein:

n is 1 or 2;

R_1 and R_2 are, independently, hydrogen atoms, hydroxyl groups, C_{1-6} alkyl groups, C_{1-6} alkenyl groups, C_{1-6} alkynyl groups, C_{1-6} alkoxyl groups, C_{1-6} aminoalkoxyl groups, halogen atoms, nitro groups, cyano groups, mercapto groups, C_{1-6} alkylthio groups, C_{1-6} hydroxyalkyl groups, C_{1-6} halogenoalkyl groups, C_{1-6} cyanoalkyl gropus, C_{1-6} nitroalkyl groups, amino groups which may contain protective groups, C_{1-6} aminoalkyl groups which may contain protective groups or C_{1-6} alkyl groups which may contain protective groups or C_{1-6} alkyl groups, C_{1-6} aminoalkylamino groups which may contain protective groups or C_{1-6} alkyl groups at the amino-position, heterocyclic C_{1-6} alkyl groups which may contain C_{1-6} alkyl, C_{1-6} alkoxyl, amino, halogeno, nitro, or cyano groups, heterocyclic C_{1-6} alkylamino groups which may contain C_{1-6} alkyl, C_{1-6} alkoxyl, amino (which may contain protective groups), halogeno, nitro, cyano groups, or protective groups, amino-heterocyclic groups which may contain protective groups or C_{1-6} alkyl groups at the nitrogen atom of the heterocyclic ring moiety or amino position, heterocyclic-amino groups which may contain protective groups of C_{1-6} alkyl groups at the nitrogen atom of the heterocyclic ring moiety or amino position, carbamoyl groups which may contain protective groups or C_{1-6} alkyl groups, heterocyclic carbonyl groups which may contain C_{1-6} alkyl, C_{1-6} alkoxyl, amino, hydroxyl, halogeno, nitro, or cyano groups;

R_3 represents an C_{1-6} alkyl group;

Z represents O.S. CH-R_4 (R_4 stands for a hydrogen atom or a C_{1-6} alkyl group), or N-R_5 (R_5 stands for a hydrogen atom, a C_{1-6} alkyl group, or a protective group for the amino group); and

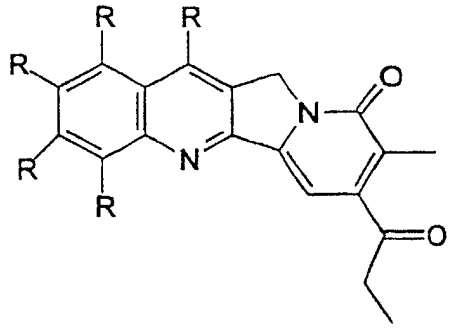
m and n independently represent 0, 1 or 2 provided that m and n are not both equal to 2, and wherein said heterocyclic group is selected from the group consisting of azetidine, pyrrolidine, piperidine, piperazine, imidazoline, and morpholine, and wherein said protective group is selected from the group consisting of acetyl, formyl, trityl, ter-butoxycarbonyl, and p-methoxybenzoyloxycarbonyl.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are

described in U.S. Patent Number 4,939,255 and U.S. Patent Number 5,062,795.

Example 12

Camptothecin analogs may have the formula:

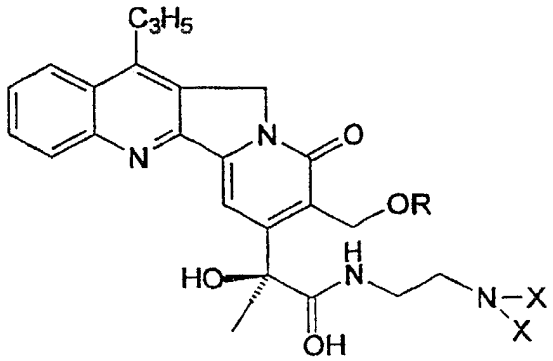


wherein the compound is 8-methyl-7-(1-oxopropyl)indolizino [1,2-b]quinolin-9(11H)-one or a substituted 8-methyl-7-(1-oxopropyl)indolizino [1,2-b]quinolin-9(11H)-one.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 5,155,225.

Example 13

Camptothecin analogs may have the formula:



wherein:

X is a lower alkyl group; and

R is a hydrogen atom or the grouping -COY where Y is a linear or branched unsubstituted C_1-C_1 alkyl group, a lower alkyl group substituted by a halogen atom or a lower alkylthio, amino, acylamino, hydroxyl, lower alkoxy, arloxy or lower alkoxycarbonyl group; a C_3-C_{19} alkenyl, C_3-C_{19} alkynyl or C_3-C_8 cycloalkyl group; a C_3-C_8 cycloalkyl group substituted by an acylamino-lower alkyl group; an N-acylpyrrolidyl group, a phenyl group; a phenyl group substituted by a halogen atom or a trifluoromethyl, nitro, amino, lower alkoxycarbonyl, lower alkyl, phenyl or lower alkoxy; a cinnamyl group; a benzyl group; a naphthyl group; a pyridyl group; a furyl group; or a thienyl group, as well as acid addition salts and quaternary ammonium salts thereof.

The synthesis of camptothecin analogs with this formula can be carried out by those having ordinary skill in the art using synthesis schemes such as those that are described in U.S. Patent Number 4,914,205.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(I) APPLICANT: Kmiec, Eric B.
Gerhold, David L.
Strauss, Allyson Cole

(ii) TITLE OF INVENTION: Anti-fungal Agents and Methods of Identifying and Using the Same

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris
(B) STREET: One Liberty Place, 46th floor
(C) CITY: Philadelphia
(D) STATE: Pennsylvania
(E) COUNTRY: U.S.A.
(F) ZIP: 19103

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Wordperfect 6.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/485,621
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/000,399
(B) FILING DATE: 21-JUN-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: DeLuca, Mark
(B) REGISTRATION NUMBER: 33,229
(C) REFERENCE/DOCKET NUMBER: TJU-1970

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 215-568-3100
(B) TELEFAX: 215-568-3439

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3143 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 547..2889

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAATTCCTCA AACACGGTCA AAAAAATACC AACTATCTTC TGTTTCTCCC CACTCACACG      60

ACCCAACTAT TTTTTTGGTG ATGGTTTTAG GCGCGACGTT AATCATTTTT ACTATTGAGA     120

ATGATTACTC CCACATTCTA TTACACCTCA TCTTCATCTT CATCTTTCAT CTTTCACATC     180

ACTAAATATA ACCTTGCGAC CTTCACAAAT TTTTTTTTTT GACAAGCAAT CCAAAATTAC     240

AATTTTCATT TCATTTCTTT TATATATAAA AGTTTTTCAC CATTAATTTC ACCACACATC     300

TCATTAGCAA TTGGGCAAAA ATAGAAAGTA ATTTTATAAC TTATAACCAA AAACAATTCA     360

AGAACAATAT CATTATTATT AAATTTATCA CGGAATTTGT TTTGCAAATC AAGTAAGAAC     420

AATTTCCATC AATTTACTCA TCAGTTTGGT TGTAATAATA AAAACAGATT ATTTTTCTTA     480

TCATCACCAC CAAGAGTATT CCGTTATTTA AATCCATTAT TTGTTCGTTC ATATAGCATA     540

ATTCCT ATG AGT TCA TCA GAC GAA GAA GAC ATT GCC TTG TCT AGA CTC         588
       Met Ser Ser Ser Asp Glu Glu Asp Ile Ala Leu Ser Arg Leu
         1               5                  10

GCT AAA AAA TCA TCC TCG ATC ACT TCA GCT TCC ACT TAT GAA GAC GAT       636
Ala Lys Lys Ser Ser Ser Ile Thr Ser Ala Ser Thr Tyr Glu Asp Asp
 15                  20                  25                  30

GAA GAT GAT GAT ATC CCT TTA GCT AAA AAA TCC AGG AAA AAG AGG GTT       684
Glu Asp Asp Asp Ile Pro Leu Ala Lys Lys Ser Arg Lys Lys Arg Val
                 35                  40                  45

GAA TCT GAT TAT GAA GAA GAT GAA GAC GAA GTC CCA TTG AAA AAG AGA       732
Glu Ser Asp Tyr Glu Glu Asp Glu Asp Glu Val Pro Leu Lys Lys Arg
             50                  55                  60

AAA TTG TCT AAT GGT AGA GCA AAA AAA CAA GTT AAA ACC GAA ACT AAA       780
Lys Leu Ser Asn Gly Arg Ala Lys Lys Gln Val Lys Thr Glu Thr Lys
         65                  70                  75

GTT AAA AAG GAA CCT AAA AGT GCC AAT AAA TCC AAA TCT ACA TCT AAA       828
Val Lys Lys Glu Pro Lys Ser Ala Asn Lys Ser Lys Ser Thr Ser Lys
     80                  85                  90

AAG GAC ACC AAA GTT AAG AAA GAG AAA ACT ACA GTC AAG AAG GAA TCT       876
Lys Asp Thr Lys Val Lys Lys Glu Lys Thr Thr Val Lys Lys Glu Ser
 95                  100                 105                 110

AAA GCC ACA AGC ACT AAA GTG AAA GAA GAA TCC AAA ACT CAA TCA GAT       924
Lys Ala Thr Ser Thr Lys Val Lys Glu Glu Ser Lys Thr Gln Ser Asp
                115                 120                 125

TCA CAA GCA TCG GTT AAA TCT GAA ACT CCT GAA GAA GAT CAA GGG TAC       972
Ser Gln Ala Ser Val Lys Ser Glu Thr Pro Glu Glu Asp Gln Gly Tyr
            130                 135                 140

AAA TGG TGG GAA GTG AAT CAA GAA GAA GAA GGT GAT GGT TAT ATC AAA      1020
Lys Trp Trp Glu Val Asn Gln Glu Glu Glu Gly Asp Gly Tyr Ile Lys
        145                 150                 155

TGG CAA ACA CTA GAA CAT AAC GGT GTT ATG TTT CCA CCA CCA TAT GAA      1068
Trp Gln Thr Leu Glu His Asn Gly Val Met Phe Pro Pro Pro Tyr Glu
    160                 165                 170

CCA TTA CCA TCT CAT GTC AAA TTA TAT TAT AAC AAT AAA CCA GTT AAT      1116
Pro Leu Pro Ser His Val Lys Leu Tyr Tyr Asn Asn Lys Pro Val Asn
```

```
175                 180                 185                 190

TTA CCT CCA GAA GCA GAA GAA GTT GCC GGA TTT TAT GGA GCA ATG TTA      1164
Leu Pro Pro Glu Ala Glu Glu Val Ala Gly Phe Tyr Gly Ala Met Leu
                195                 200                 205

GAA ACT GAT CAT GCT AAA AAC CCA GTT TTC CAA AAG AAT TTT TTC AAT      1212
Glu Thr Asp His Ala Lys Asn Pro Val Phe Gln Lys Asn Phe Phe Asn
            210                 215                 220

GAT TTT TTG GAA GTT TTA AAA GAA TGT GGT GGT TGT GGT GTT GAA ATT      1260
Asp Phe Leu Glu Val Leu Lys Glu Cys Gly Gly Cys Gly Val Glu Ile
        225                 230                 235

AAA AAA TTT GAA AAA TTA GAT TTT AGT AAA ATG TAT GCT CAT TTT GAA      1308
Lys Lys Phe Glu Lys Leu Asp Phe Ser Lys Met Tyr Ala His Phe Glu
    240                 245                 250

AAA TTA CGT GAA GAG AAA AAG GCC ATG AGT AGG GAA GAA AAG AAA AGA      1356
Lys Leu Arg Glu Glu Lys Lys Ala Met Ser Arg Glu Glu Lys Lys Arg
255                 260                 265                 270

ATC AAA GAA GAA AAA GAA AAA GAA GAA GAA CCT TAT AGG ACT TGT TAT      1404
Ile Lys Glu Glu Lys Glu Lys Glu Glu Glu Pro Tyr Arg Thr Cys Tyr
                275                 280                 285

CTT AAT GGT AGA AAA GAA TTA GTG GGG AAT TTC CGT ATT GAA CCT CCA      1452
Leu Asn Gly Arg Lys Glu Leu Val Gly Asn Phe Arg Ile Glu Pro Pro
            290                 295                 300

GGT TTA TTC CGT GGT CGT GGT GCA CAT CCT AAA ACT GGG AAA TTA AAA      1500
Gly Leu Phe Arg Gly Arg Gly Ala His Pro Lys Thr Gly Lys Leu Lys
        305                 310                 315

CGT CGA GTA GTG CTG GAA CAA GTG ACT TTG AAT TTA GGT AAA GAT GCT      1548
Arg Arg Val Val Leu Glu Gln Val Thr Leu Asn Leu Gly Lys Asp Ala
    320                 325                 330

AAA ATA CCT GAA CCA CCT GCA GGC CAT CAA TGG GGG GAA ATT AGA CAT      1596
Lys Ile Pro Glu Pro Pro Ala Gly His Gln Trp Gly Glu Ile Arg His
335                 340                 345                 350

GAT AAT GAA GTC ACT TGG TTA GCC ATG TGG AAA GAA AAT ATT TCT GAT      1644
Asp Asn Glu Val Thr Trp Leu Ala Met Trp Lys Glu Asn Ile Ser Asp
                355                 360                 365

TCA TTG AAA TAC GTT AGA TTT GCT AAT AAT TCT TCA GTT AAA GGT CAA      1692
Ser Leu Lys Tyr Val Arg Phe Ala Asn Asn Ser Ser Val Lys Gly Gln
            370                 375                 380

TCC GAT TTC AAA AAA TTT GAA ACG GCG AGA AAA TTA AGA GAT CAC GTT      1740
Ser Asp Phe Lys Lys Phe Glu Thr Ala Arg Lys Leu Arg Asp His Val
        385                 390                 395

GAT TCT ATT AGA AAA GAT TAT ACC AAA ATG TTA AAA TCA GAG AAA ATG      1788
Asp Ser Ile Arg Lys Asp Tyr Thr Lys Met Leu Lys Ser Glu Lys Met
    400                 405                 410

CAA GAT AGA CAA ATG GCC ACG GCT ATG TAT CTT ATT GAT GTT TTT GCA      1836
Gln Asp Arg Gln Met Ala Thr Ala Met Tyr Leu Ile Asp Val Phe Ala
415                 420                 425                 430

TTG AGG GCT GGT GGT GAA AAA GGT GAG GAT GAA GCC GAT ACC GTT GGT      1884
Leu Arg Ala Gly Gly Glu Lys Gly Glu Asp Glu Ala Asp Thr Val Gly
                435                 440                 445

TGT TGT TCA TTA CGA TAT GAA CAT GTA ACT TTA AAA CCA CCC AAC AAG      1932
```

```
Cys Cys Ser Leu Arg Tyr Glu His Val Thr Leu Lys Pro Pro Asn Lys
                450                 455                 460

GTT ATT TTC GAT TTT TTG GGT AAA GAT TCA ATT AGA TTT TAT CAA GAA      1980
Val Ile Phe Asp Phe Leu Gly Lys Asp Ser Ile Arg Phe Tyr Gln Glu
        465                 470                 475

GTT GAA GTT GAT AAA CAA GTT TTC AAA AAT CTA CGA ATT TTC AAA AAA      2028
Val Glu Val Asp Lys Gln Val Phe Lys Asn Leu Arg Ile Phe Lys Lys
    480                 485                 490

TCT CCT AAA CAA CCT GGT GAT GAT TTA TTT GAT CGT ATA AAC CCT TCA      2076
Ser Pro Lys Gln Pro Gly Asp Asp Leu Phe Asp Arg Ile Asn Pro Ser
495                 500                 505                 510

TTA GTC AAT CGA CAA TTA CAA AAT TAT ATG AAA GGA TTA ACA GCA AAA      2124
Leu Val Asn Arg Gln Leu Gln Asn Tyr Met Lys Gly Leu Thr Ala Lys
                515                 520                 525

GTT TTC CGT ACA TAT AAT GCC TCG AAA ACC ATG CAA GAT CAA ATT GAT      2172
Val Phe Arg Thr Tyr Asn Ala Ser Lys Thr Met Gln Asp Gln Ile Asp
                530                 535                 540

ATA ATT GAA AAT GAA GGT ACA GTG GCG GAA AAA GTG GCT AAA TTC AAT      2220
Ile Ile Glu Asn Glu Gly Thr Val Ala Glu Lys Val Ala Lys Phe Asn
        545                 550                 555

GCT GCC AAT AGA ACG GTG GCT ATT TTA TGT AAT CAC CAG CGT ACG GTC      2268
Ala Ala Asn Arg Thr Val Ala Ile Leu Cys Asn His Gln Arg Thr Val
    560                 565                 570

AGT AAA ACC CAT GGT GAT AGT GTT CAG AGA ATT AAT GAC AAA TTG AAA      2316
Ser Lys Thr His Gly Asp Ser Val Gln Arg Ile Asn Asp Lys Leu Lys
575                 580                 585                 590

AAA TTC ATG TGG CAA AAG ATT AGA TTA AAG AAA ATG ATC TTA CAA TTA      2364
Lys Phe Met Trp Gln Lys Ile Arg Leu Lys Lys Met Ile Leu Gln Leu
                595                 600                 605

GAA CCC AAA TTG AAA AAG AAA GAT TCG AAA TAT TTT GAA GAA ATT GAT      2412
Glu Pro Lys Leu Lys Lys Lys Asp Ser Lys Tyr Phe Glu Glu Ile Asp
                610                 615                 620

GAT TTA CTC AAA GAA GAT ATT GAA CAT ATT CAT CAT ACT ATA ATT AAA      2460
Asp Leu Leu Lys Glu Asp Ile Glu His Ile His His Thr Ile Ile Lys
        625                 630                 635

AGA CAA CGA GAA CAA GCT AAA AAA AAA TTA GAA CGT GAT AAT GAA AAA      2508
Arg Gln Arg Glu Gln Ala Lys Lys Lys Leu Glu Arg Asp Asn Glu Lys
    640                 645                 650

TTG AAA CTT GAA GGT AAA CCA TTA TTA ACT GAA TCA GAT ATA AAA GAT      2556
Leu Lys Leu Glu Gly Lys Pro Leu Leu Thr Glu Ser Asp Ile Lys Asp
655                 660                 665                 670

AAA TTA GAT AAA ATT GAT GAA TTA GAA AAA GAA TAT CAA AAA GAA TTG      2604
Lys Leu Asp Lys Ile Asp Glu Leu Glu Lys Glu Tyr Gln Lys Glu Leu
                675                 680                 685

AAA ACT GGT AAA CCA ATA GTC ACC AAA AAT GCT ACC GTT GAA AAA TTA      2652
Lys Thr Gly Lys Pro Ile Val Thr Lys Asn Ala Thr Val Glu Lys Leu
                690                 695                 700

AAA CAA CAA ATT GAA ACT CTT GAA AAT AAA ATT CTT AAT GTT TCA ATT      2700
Lys Gln Gln Ile Glu Thr Leu Glu Asn Lys Ile Leu Asn Val Ser Ile
        705                 710                 715
```

```
CAA TTA AAA GAT AAA GAA GAT AAT TCT GAA GTT TCT TTA GGA ACT TCA      2748
Gln Leu Lys Asp Lys Glu Asp Asn Ser Glu Val Ser Leu Gly Thr Ser
    720                 725                 730

AAA ATG AAT TAT ATT GAT CCA AGA TTA ATT GTT ATG TTT TCT AAA AAA      2796
Lys Met Asn Tyr Ile Asp Pro Arg Leu Ile Val Met Phe Ser Lys Lys
735                 740                 745                 750

TTT GAT GTT CCT ATT GAA AAA TTA TTT ACC AAA ACT TTA AGA GAA AAG      2844
Phe Asp Val Pro Ile Glu Lys Leu Phe Thr Lys Thr Leu Arg Glu Lys
                755                 760                 765

TTC ATT TGG GCT ATT GAA TCA GCT GAT GAA AAT TGG AGA TTC TAA          2889
Phe Ile Trp Ala Ile Glu Ser Ala Asp Glu Asn Trp Arg Phe  *
            770                 775                 780

AATTAGGGGT TTGTTTCTTA GCTTATTATT ATATACTATA TGCTGTAGAG TAAAATTTTG    2949

TACCTTGTAA TATATATATA TACATTGTTT CAACATAGAA AAATAGATTG ATACTGCAGT    3009

ATGAAAAAGA ATATGCACAC ACCAAGCAAG TGTATTTTAG ATAAAGGATT GGTGTTTTGA    3069

TATTGGAAGG GTGAAAGATG AAGGGGGTAT CACACAGACA CGTACAATCA AGAAATTGAA    3129

ATTTCTCCGA ATTC                                                      3143
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 781 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ser Ser Ser Asp Glu Glu Asp Ile Ala Leu Ser Arg Leu Ala Lys
 1               5                  10                  15

Lys Ser Ser Ser Ile Thr Ser Ala Ser Thr Tyr Glu Asp Asp Glu Asp
            20                  25                  30

Asp Asp Ile Pro Leu Ala Lys Lys Ser Arg Lys Lys Arg Val Glu Ser
        35                  40                  45

Asp Tyr Glu Glu Asp Glu Asp Glu Val Pro Leu Lys Lys Arg Lys Leu
    50                  55                  60

Ser Asn Gly Arg Ala Lys Lys Gln Val Lys Thr Glu Thr Lys Val Lys
65                  70                  75                  80

Lys Glu Pro Lys Ser Ala Asn Lys Ser Lys Ser Thr Ser Lys Lys Asp
                85                  90                  95

Thr Lys Val Lys Lys Glu Lys Thr Thr Val Lys Lys Glu Ser Lys Ala
            100                 105                 110

Thr Ser Thr Lys Val Lys Glu Glu Ser Lys Thr Gln Ser Asp Ser Gln
        115                 120                 125

Ala Ser Val Lys Ser Glu Thr Pro Glu Glu Asp Gln Gly Tyr Lys Trp
    130                 135                 140

Trp Glu Val Asn Gln Glu Glu Glu Gly Asp Gly Tyr Ile Lys Trp Gln
145                 150                 155                 160
```

Thr Leu Glu His Asn Gly Val Met Phe Pro Pro Pro Tyr Glu Pro Leu
165 170 175

Pro Ser His Val Lys Leu Tyr Tyr Asn Asn Lys Pro Val Asn Leu Pro
180 185 190

Pro Glu Ala Glu Glu Val Ala Gly Phe Tyr Gly Ala Met Leu Glu Thr
195 200 205

Asp His Ala Lys Asn Pro Val Phe Gln Lys Asn Phe Phe Asn Asp Phe
210 215 220

Leu Glu Val Leu Lys Glu Cys Gly Gly Cys Gly Val Glu Ile Lys Lys
225 230 235 240

Phe Glu Lys Leu Asp Phe Ser Lys Met Tyr Ala His Phe Glu Lys Leu
245 250 255

Arg Glu Glu Lys Lys Ala Met Ser Arg Glu Glu Lys Lys Arg Ile Lys
260 265 270

Glu Glu Lys Glu Lys Glu Glu Glu Pro Tyr Arg Thr Cys Tyr Leu Asn
275 280 285

Gly Arg Lys Glu Leu Val Gly Asn Phe Arg Ile Glu Pro Pro Gly Leu
290 295 300

Phe Arg Gly Arg Gly Ala His Pro Lys Thr Gly Lys Leu Lys Arg Arg
305 310 315 320

Val Val Leu Glu Gln Val Thr Leu Asn Leu Gly Lys Asp Ala Lys Ile
325 330 335

Pro Glu Pro Pro Ala Gly His Gln Trp Gly Glu Ile Arg His Asp Asn
340 345 350

Glu Val Thr Trp Leu Ala Met Trp Lys Glu Asn Ile Ser Asp Ser Leu
355 360 365

Lys Tyr Val Arg Phe Ala Asn Asn Ser Ser Val Lys Gly Gln Ser Asp
370 375 380

Phe Lys Lys Phe Glu Thr Ala Arg Lys Leu Arg Asp His Val Asp Ser
385 390 395 400

Ile Arg Lys Asp Tyr Thr Lys Met Leu Lys Ser Glu Lys Met Gln Asp
405 410 415

Arg Gln Met Ala Thr Ala Met Tyr Leu Ile Asp Val Phe Ala Leu Arg
420 425 430

Ala Gly Gly Glu Lys Gly Glu Asp Glu Ala Asp Thr Val Gly Cys Cys
435 440 445

Ser Leu Arg Tyr Glu His Val Thr Leu Lys Pro Pro Asn Lys Val Ile
450 455 460

Phe Asp Phe Leu Gly Lys Asp Ser Ile Arg Phe Tyr Gln Glu Val Glu
465 470 475 480

Val Asp Lys Gln Val Phe Lys Asn Leu Arg Ile Phe Lys Lys Ser Pro
485 490 495

Lys Gln Pro Gly Asp Asp Leu Phe Asp Arg Ile Asn Pro Ser Leu Val
500 505 510

Asn Arg Gln Leu Gln Asn Tyr Met Lys Gly Leu Thr Ala Lys Val Phe

```
        515                 520                 525

Arg Thr Tyr Asn Ala Ser Lys Thr Met Gln Asp Gln Ile Asp Ile Ile
    530                 535                 540

Glu Asn Glu Gly Thr Val Ala Glu Lys Val Ala Lys Phe Asn Ala Ala
545                 550                 555                 560

Asn Arg Thr Val Ala Ile Leu Cys Asn His Gln Arg Thr Val Ser Lys
                565                 570                 575

Thr His Gly Asp Ser Val Gln Arg Ile Asn Asp Lys Leu Lys Lys Phe
            580                 585                 590

Met Trp Gln Lys Ile Arg Leu Lys Lys Met Ile Leu Gln Leu Glu Pro
        595                 600                 605

Lys Leu Lys Lys Lys Asp Ser Lys Tyr Phe Glu Glu Ile Asp Asp Leu
    610                 615                 620

Leu Lys Glu Asp Ile Glu His Ile His His Thr Ile Ile Lys Arg Gln
625                 630                 635                 640

Arg Glu Gln Ala Lys Lys Lys Leu Glu Arg Asp Asn Glu Lys Leu Lys
                645                 650                 655

Leu Glu Gly Lys Pro Leu Leu Thr Glu Ser Asp Ile Lys Asp Lys Leu
            660                 665                 670

Asp Lys Ile Asp Glu Leu Glu Lys Glu Tyr Gln Lys Glu Leu Lys Thr
        675                 680                 685

Gly Lys Pro Ile Val Thr Lys Asn Ala Thr Val Glu Lys Leu Lys Gln
    690                 695                 700

Gln Ile Glu Thr Leu Glu Asn Lys Ile Leu Asn Val Ser Ile Gln Leu
705                 710                 715                 720

Lys Asp Lys Glu Asp Asn Ser Glu Val Ser Leu Gly Thr Ser Lys Met
                725                 730                 735

Asn Tyr Ile Asp Pro Arg Leu Ile Val Met Phe Ser Lys Lys Phe Asp
            740                 745                 750

Val Pro Ile Glu Lys Leu Phe Thr Lys Thr Leu Arg Glu Lys Phe Ile
        755                 760                 765

Trp Ala Ile Glu Ser Ala Asp Glu Asn Trp Arg Phe  *
    770                 775                 780
```

CLAIMS

1. A substantially pure protein having the amino acid sequence of SEQ ID NO:2.

2. An isolated nucleic acid molecule consisting of SEQ ID NO:1 or a fragment thereof having at least 10 nucleotides.

3. The nucleic acid molecule of claim 2 consisting of SEQ ID NO:1.

4. A recombinant expression vector comprising a nucleic acid sequence that encodes the protein of claim 1.

5. A host cell comprising the recombinant expression vector of claim 4.

6. A recombinant expression vector comprising the nucleic acid molecule of claim 3.

7. A host cell comprising the recombinant expression vector of claim 6.

8. The nucleic acid molecule of claim 2 consisting of a fragment of SEQ ID NO:1 having at least 10 nucleotides.

9. The nucleic acid molecule of claim 2 consisting of a fragment of SEQ ID NO:1 having 12-150 nucleotides.

10. The nucleic acid molecule of claim 2 consisting of a fragment of SEQ ID NO:1 having 15-50 nucleotides.

11. The nucleic acid molecule of claim 2 consisting of a fragment of SEQ ID NO:1 having 18-30 nucleotides.

12. The nucleic acid molecule of claim 2 consisting of a fragment of SEQ ID NO:1 having 24 nucleotides.

13. An oligonucleotide molecule comprising a nucleotide sequence complimentary to a nucleotide sequence of at least 10 nucleotides of SEQ ID NO:1.

14. The oligonucleotide molecule of claim 13 consisting of a nucleotide sequence complimentary to a nucleotide sequence of at least 10-150 nucleotides of SEQ ID NO:1.

15. The oligonucleotide molecule of claim 14 consisting of a nucleotide sequence complimentary to a nucleotide sequence of at least 18-28 nucleotides of SEQ ID NO:1.

16. An isolated antibody which binds to an epitope on SEQ ID NO:2.

17. The antibody of claim 16 which binds to an epitope that includes amino acids 730 to 740 on SEQ ID NO:2.

18. The antibody of claim 16 wherein said antibody is a monoclonal antibody.

19. A method of identifying inhibitors of *C. albicans* topoisomerase I protein comprising the steps of:

contacting a first host cell which is deficient in a functional topoisomerase gene except for a functional gene that encodes *C. albicans* topoisomerase I protein with a test compound;

contacting a second host cell which is deficient in a functional topoisomerase gene except for a functional gene that encodes *non-C. albicans* topoisomerase I protein with a test compound;

identifying a test compound whose presence results in the death of the first host cell but not the second host cell.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09530

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 9/90
US CL : 435/233
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/233, 922

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog files 155, 5, 434, 350, 351 (MEDLINE, BIOSIS, SCISEARCH, DERWENT WPI), APS
search terms: topoisomerase, candida

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	TAYLOR et al. Identification of the gene encoding DNA topoisomerase I from Candida albicans. FEMS Microbiology Letters, 01 May 1996, Vol. 138, No. 2-3, pages 113-121, especially figure 3.	1
X	FOSTEL et al. Characterization of DNA topoisomerase I from Candida albicans as a target for drug discovery. Antimicrob. Agents Chemotherap. October 1992, Vol. 36, Number 10, pages 2131-2138, especially page 2132.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents.	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
16 JULY 1996	14 AUG 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	GABRIELE E. SUGAISKY
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09530

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09530

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

I. Claim 1, drawn to C. albicans topoisomerase I.
II. Claims 2-15, drawn to DNA encoding C. albicans topoisomerase I, vectors and host cells containing the gene.
III. Claims 16-18, drawn to antibodies specific for C.albicans topoisomerase I.
IV. Claim 19, drawn to a method of identifying topoisomerase inhibitors using cells transformed with a gene encoding C. albicans topoisomerase I.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The common technical feature is C. albicans topoisomerase I; however, this was known in the art before the priority date of the instant application. Accordingly, it does not constitute a special technical feature linking all claims as a single contribution over the art, and a holding of lack of unity is therefore proper.